

Amendment to the Claims:

Please amend the claims as follows.

This listing of claims will replace all prior versions, and listing, of claims in the application:

Listing of Claims:

Claim 1 (currently amended): An isolated, synthetic or recombinant nucleic acid comprising (a) a sequence having at least 90% sequence identity to SEQ ID NO:26, and encoding a polypeptide having an esterase activity, or, (b) a sequence complementary to (a).

Claim 2 (currently amended): An isolated, synthetic or recombinant nucleic acid of claim 1, comprising a sequence comprising SEQ ID NO:26 or sequences complementary thereto.

Claim 3 (currently amended): An isolated, synthetic or recombinant nucleic acid (a) encoding a polypeptide having an esterase activity comprising a sequence that hybridizes to the [[a]] nucleic acid of comprising (a) a sequence as set forth in SEQ ID NO:26, and encoding a polypeptide having an esterase activity, or (b) sequences complementary to (a),

wherein the hybridization under conditions comprise comprising about 50% formamide at about 37°C to 42°C, 5X SSPE, 0.3% SDS, and 200 ng/ml sheared and denatured salmon sperm DNA, and a wash for 30 minutes at room temperature in 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na<sub>2</sub>EDTA containing 0.5% SDS.

Claim 4 (currently amended): An isolated, synthetic or recombinant nucleic acid (a) encoding a polypeptide having an esterase activity comprising a sequence that hybridizes to the [[a]] nucleic acid of comprising (a) a sequence as set forth in SEQ ID NO:26, and encoding a polypeptide having an esterase activity, or (b) sequences complementary to (a),

wherein under hybridization conditions comprise comprising a wash for 30 minutes at room temperature in 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na<sub>2</sub>EDTA containing 0.5% SDS, followed by a 30 minute wash in fresh 1X SET at T<sub>m</sub>-10°C.

Claim 5 (currently amended): An isolated, synthetic or recombinant nucleic acid (a) encoding a polypeptide having an esterase activity comprising a sequence that hybridizes to the [[a]] nucleic acid of comprising (a) a sequence as set forth in SEQ ID NO:26, and encoding a polypeptide having an esterase activity, or (b) sequences complementary to (a),

wherein the hybridization under conditions comprise comprising about 35% formamide at about 35°C to 42°C, 5X SSPE, 0.3% SDS, and 200 ng/ml sheared and denatured salmon sperm DNA.

Claims 6 to 15 (canceled)

Claim 16 (currently amended): An isolated, synthetic or recombinant nucleic acid comprising (a) at least 30 consecutive bases of a sequence as set forth in SEQ ID NO:26, and encoding a polypeptide having an esterase activity, or (b) sequences complementary to (a), ~~wherein the nucleic acid can specifically hybridize to an esterase encoding sequence under hybridization conditions comprising a wash for 30 minutes at room temperature in 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na<sub>2</sub>EDTA containing 0.5% SDS, followed by a 30 minute wash in fresh 1X SET at T<sub>m</sub>-10°C.~~

Claims 17 to 19 (canceled)

Claim 20 (currently amended): The isolated, synthetic or recombinant nucleic acid of claim 1, wherein the nucleic acid has at least [[a]] 95% sequence identity to SEQ ID NO:26.

Claim 21 (currently amended): The isolated, synthetic or recombinant nucleic acid of claim 20, wherein the sequence identity to SEQ ID NO:26 is at least about 97%.

Claim 22 (currently amended): An isolated, synthetic or recombinant nucleic acid comprising a sequence (i) encoding (a) a polypeptide having an esterase activity and having at least 90% sequence identity to the [[a]] sequence of as set forth in SEQ ID NO:36, or, (b) enzymatically active fragments of (a); or (ii) fully complementary to (i).

Claim 23 (currently amended): An isolated, synthetic or recombinant nucleic acid comprising a sequence (i) encoding (a) a polypeptide having an esterase activity comprising at least 30 consecutive amino acids of a polypeptide ~~having an esterase activity and~~ having at least 90% sequence identity to the [[a]] sequence of ~~as set forth in~~ SEQ ID NO:36; or (ii) fully complementary to (i).

Claims 24 to 39 (canceled)

Claim 40 (previously presented): A method of producing a polypeptide having an esterase activity comprising introducing a nucleic acid as set forth in claim 1 or claim 3 into a host cell under conditions that allow expression of the nucleic acid to produce a polypeptide.

Claim 41 (currently amended): A method of producing a polypeptide having esterase activity comprising at least 30 amino acids of the amino acid [[a]] sequence of ~~as set forth in~~ SEQ ID NO:36 or at least 30 amino acids of a sequence encoded by the [[a]] nucleic acid sequence of as set forth in claim 1, comprising introducing a nucleic acid encoding the polypeptide, operably linked to a promoter, into a host cell under conditions that allow expression of the polypeptide, wherein said polypeptide has esterase activity.

Claim 42 (withdrawn): A method of generating a variant comprising:  
obtaining a nucleic acid comprising a sequence as set forth in SEQ ID NO:26, or a sequence as set forth in claim 1, or, sequences complementary thereto, or fragments comprising at least 30 consecutive nucleotides thereof, or fragments comprising at least 30 consecutive nucleotides of the sequences complementary to SEQ ID NO:26; and  
modifying one or more nucleotides in said sequence to another nucleotide, deleting one or more nucleotides in said sequence, or adding one or more nucleotides to said sequence.

Claim 43 (withdrawn): The method of claim 42, wherein the modifications are introduced by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis,

recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturation mutagenesis and any combination thereof.

Claim 44 (withdrawn): The method of claim 42, wherein the modifications are introduced by error-prone PCR.

Claim 45 (withdrawn): The method of claim 42, wherein the modifications are introduced by shuffling.

Claim 46 (withdrawn): The method of claim 42, wherein the modifications are introduced by oligonucleotide-directed mutagenesis.

Claim 47 (withdrawn): The method of claim 42, wherein the modifications are introduced by assembly PCR.

Claim 48 (withdrawn): The method of claim 42, wherein the modifications are introduced by sexual PCR mutagenesis.

Claim 49 (withdrawn): The method of claim 42, wherein the modifications are introduced by *in vivo* mutagenesis.

Claim 50 (withdrawn): The method of claim 42, wherein the modifications are introduced by cassette mutagenesis.

Claim 51 (withdrawn): The method of claim 42, wherein the modifications are introduced by recursive ensemble mutagenesis.

Claim 52 (withdrawn): The method of claim 42, wherein the modifications are introduced by exponential ensemble mutagenesis.

Claim 53 (withdrawn): The method of claim 42, wherein the modifications are introduced by site-specific mutagenesis.

Claim 54 (withdrawn): The method of claim 42, wherein the modifications are introduced by gene reassembly.

Claim 55 (withdrawn): The method of claim 42, wherein the modifications are introduced by gene site gene site saturation mutagenesis.

Claims 56 to 60 (canceled)

Claim 61 (withdrawn): A method for comparing a first sequence to a reference sequence wherein said first sequence comprises a nucleic acid sequence as set forth in claim 1 or claim 3, the method comprising the following steps:

reading the first sequence and the reference sequence through use of a computer program which compares sequences; and

determining differences between the first sequence and the reference sequence with the computer program.

Claim 62 (withdrawn): The method of claim 61, wherein determining differences between the first sequence and the reference sequence comprises identifying polymorphisms.

Claim 63 (withdrawn): A method for identifying a feature in a sequence as set forth in claim 1 or claim 3, the method comprising the following steps:

reading the sequence through the use of a computer program which identifies features in sequences; and

identifying features in the sequences with the computer program.

Claim 64 (canceled)

Claim 65 (withdrawn): A method of catalyzing the hydrolysis of an ester comprising contacting a sample containing an esterase with a polypeptide encoded by a sequence as set forth in claim 1 under conditions which facilitate the hydrolysis of the ester.

Claim 66 (canceled)

Claim 67 (currently amended): A nucleic acid probe for isolation or identification of esterase genes comprising an oligonucleotide at least 30 nucleotides in length and having ~~an area of~~ at least 30 contiguous nucleotides of the [[a]] nucleic acid sequence of claim 4 as set forth in SEQ ID NO:26 or (b) a sequence complementary to (a), ~~wherein the nucleic acid probe can specifically hybridize to an esterase encoding sequence under hybridization conditions comprising a wash for 30 minutes at room temperature in 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na<sub>2</sub>EDTA containing 0.5% SDS, followed by a 30 minute wash in fresh 1X SET at T<sub>m</sub>-10°C.~~

Claim 68 (previously presented): The probe of claim 67, wherein the oligonucleotide comprises DNA or RNA.

Claims 69 to 79 (canceled)

Claim 80 (original): The probe of claim 67, wherein the probe further comprises a detectable isotopic label.

Claim 81 (original): The probe of claim 67, wherein the probe further comprises a detectable non-isotopic label selected from the group consisting of a fluorescent molecule, a chemiluminescent molecule, an enzyme, a cofactor, an enzyme substrate, and a hapten.

Claim 82 (currently amended): A nucleic acid probe for isolation or identification of esterase genes comprising an oligonucleotide at least about 20 nucleotides in length and having ~~an area of~~ at least 20 contiguous nucleotides of a sequence as set forth in SEQ ID NO:26, or, (b) its complementary sequence.

Claims 83 to 87 (canceled)

Claim 88 (withdrawn): A method for modifying small molecules, comprising mixing a polypeptide encoded by a polynucleotide of claim 1 or claim 3 thereof with a small molecule to produce a modified small molecule.

Claim 89 (withdrawn): The method of claim 88 wherein a library of modified small molecules is tested to determine if a modified small molecule is present within the library which exhibits a desired activity.

Claim 90 (withdrawn): The method of claim 89 wherein a specific biocatalytic reaction which produces the modified small molecule of desired activity is identified by systematically eliminating each of the biocatalytic reactions used to produce a portion of the library, and then testing the small molecules produced in the portion of the library for the presence or absence of the modified small molecule with the desired activity.

Claim 91 (withdrawn): The method of claim 90 wherein the specific biocatalytic reactions which produce the modified small molecule of desired activity is optionally repeated.

Claim 92 (withdrawn): The method of claim 90 or 91 wherein (a) the biocatalytic reactions are conducted with a group of biocatalysts that react with distinct structural moieties found within the structure of a small molecule, (b) each biocatalyst is specific for one structural moiety or a group of related structural moieties; and (c) each biocatalyst reacts with many different small molecules which contain the distinct structural moiety.

Claims 93 to 97 (canceled)

Claim 98 (previously presented): A vector comprising a nucleic acid as set forth in claim 1 or claim 3.

Claim 99 (previously presented): The vector of claim 98, wherein the vector comprises a viral particle, a baculovirus, a phage, a plasmid, a cosmid, a fosmid, a bacterial artificial chromosome, a viral DNA or a P1-based artificial chromosome.

Claim 100 (previously presented): A host cell comprising a nucleic acid as set forth in claim 1 or claim 3.

Claim 101 (previously presented): The host cell of claim 100 comprising a eukaryotic cell or a prokaryotic cell.

Claim 102 (previously presented): The host cell of claim 101 comprising a plant cell, a mammalian cell, a fungal cell, a bacterial cell, a yeast cell or an insect cell.

Claims 103 to 106 (canceled)

Claim 107 (currently amended): The isolated, synthetic or recombinant nucleic acid of claim 1 (a) or claim 3 (a), wherein the esterase activity comprises catalyzing the hydrolysis ~~eatalysis~~ of ~~hydrolyzing~~ an ester groups to an organic acid ~~acids~~ and an alcohol ~~aleohols~~.

Claim 108 (currently amended): The isolated, synthetic or recombinant nucleic acid of claim 4 (a) ~~1 or claim 3~~, wherein the esterase activity comprises catalyzing the hydrolysis of an ester to an organic acid and an alcohol ~~hydrolase activity~~.

Claim 109 (currently amended): The isolated, synthetic or recombinant nucleic acid of claim 1 (a) or claim 3 (a), wherein the esterase activity functions at temperatures above 100°C, or, below 0°C.

Claim 110 (previously presented): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 35 nucleotides in length.



Claim 111 (previously presented): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 40 nucleotides in length.

Claim 112 (canceled)

Claim 113 (previously presented): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 50 nucleotides in length.

Claim 114 (previously presented): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 75 nucleotides in length.

Claim 115 (previously presented): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 100 nucleotides in length.

Claim 116 (previously presented): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 150 nucleotides in length.

Claim 117 (previously presented): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 200 nucleotides in length.

Claims 118 to 125 (canceled)

Claim 126 (currently amended): The isolated, synthetic or recombinant nucleic acid of claim 1 (a) or claim 3 (a), wherein the polypeptide retains an esterase activity in an environment comprising a pH of greater than pH 11.

Claim 127 (new): The isolated, synthetic or recombinant nucleic acid of claim 21, wherein the sequence identity to SEQ ID NO:26 is at least about 98%.

Claim 128 (new): The isolated, synthetic or recombinant nucleic acid of claim 22, wherein the sequence identity to SEQ ID NO:36 is at least about 95%.

Claim 129 (new): The isolated, synthetic or recombinant nucleic acid of claim 128, wherein the sequence identity to SEQ ID NO:36 is at least about 98%.